Product Datasheet

Anti-Glypican 3 (GPC3) [IPI-mGPC3.13]

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Overview

Antigen	Glypican 3 (GPC3)
Immunogen	Purified recombinant fragment of Mouse Glypican 3 (GPC3), AA: 25-553.
Host/isotype	Rabbit/IgG1
Clonality	Recombinant monoclonal
Clone name	IPI-mGPC3.13
RRID	AB_3101861
IPI ID	TAB0010786-013-002
Specificity	GPC3; Does not recognize other GPCs
Species reactivity	Mouse and human
Amount	100 µg
Concentration	1 mg/mL
Purification	Expressed in ExpiCHO cells and affinity purified using Protein A
Storage buffer	PBS, pH 7.4 with 0.02% ProClin 300
Shipping	Shipped on blue ice at +4C
Storage	Store at +4C for up to 3 months. For long-term storage, aliquot and store at -20C. Avoid multiple freeze/thaw cycles.

IPI Tested Applications‡

Application	Tested concentration	Result	Reference
Cell Display	5 µg/mL	Positive	https://doi.org/10.57733/addgene.ohyqj9
Flow	5 µg/mL	Positive	https://doi.org/10.57733/addgene.jj5ts7
IF	1 µg/mL	Positive	https://doi.org/10.57733/addgene.3h6ynb
WB	1 µg/mL	Negative	Unpublished

‡ Not suitable for WB application.

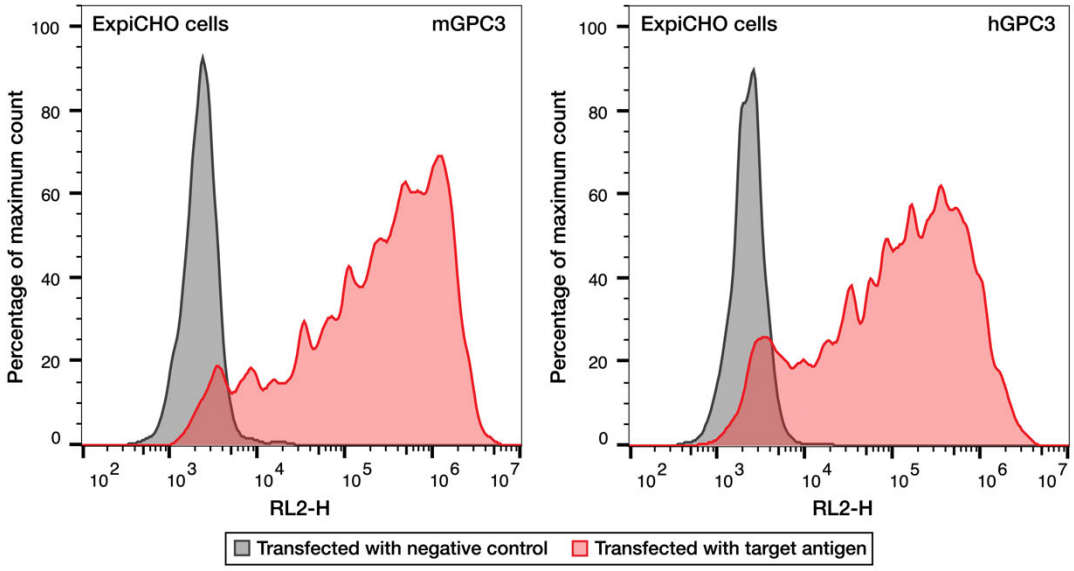
Community Data*

Application	Lab	Reference
IF	Adrian Salic, Ph.D., Harvard Medical School	https://doi.org/10.57733/addgene.9krw4c
IHC	James Trimmer, Ph.D., UC Davis	https://doi.org/10.57733/addgene.qjafi3

* Supporting Data is generated by external partner labs, in the process of evaluating IPI antibody panels.

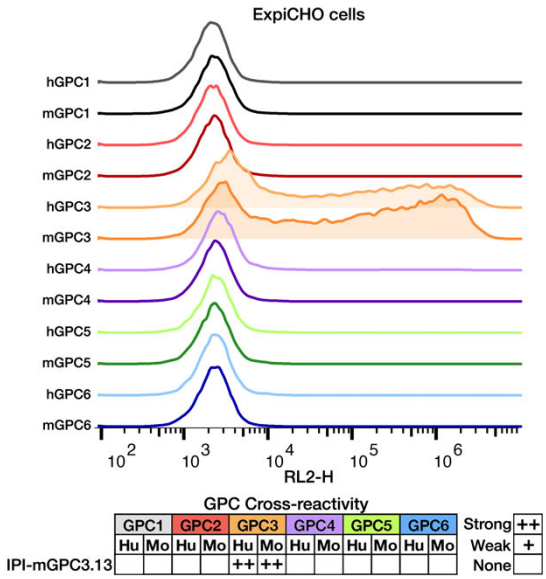
Applications

Cell Display



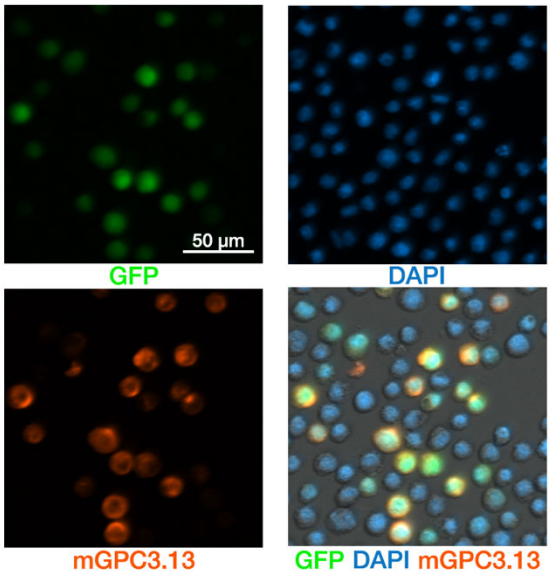
Anti-Glypican 3 (GPC3) [IPI-mGPC3.13] recognizes mouse and human GPC3. Histogram from FACS analysis on ExpiCHO cells transfected with mouse or human GPC3 (red), or WY0041 negative control (gray). Cells expressing mouse (left panel) or human (right panel) GPC3 were labeled with Anti-Glypican 3 (GPC3) [IPI-mGPC3.13] (Addgene #222514) and Alexa Fluor 647 F(ab')2 Fragment Goat Anti-Rabbit IgG Fc (Jackson ImmunoResearch, 111-606-046). Labeled cells were analyzed with an Intellicyt iQue Screener Plus flow cytometer. Histograms were generated and normalized to mode using FlowJo™ v10.10. doi: https://doi.org/10.57733/addgene.ohyqj9

Flow cytometry



Histogram overlays from FACS analysis on ExpiCHO cells transfected with human and mouse glypicans (GPC1-GPC6). Cells expressing GPCs were labeled with Anti-Glypican 3 (GPC3) [IPI-mGPC3.13] (Addgene #222514) and Alexa Fluor 647 F(ab')2 Fragment Goat Anti-Rabbit IgG Fc (Jackson ImmunoResearch, 111-606-046). Labeled cells were analyzed with an Intellicyt iQue Screener Plus flow cytometer. Anti-Glypican 3 (GPC3) [IPI-mGPC3.13] is specific for human and mouse GPC3 (orange lines with fill) and does not recognize other GPCs. Histograms were normalized to mode and displayed as an overlay with half offset using FlowJo™ v10.10. Bottom table summarizes GPC cross-reactivity. Abbreviations: glypican (GPC), human (Hu), mouse (Mo), strong (++), weak (+), none ( ). doi: https://doi.org/10.57733/addgene.jj5ts7

Immunofluorescence



Immunofluorescence (IF) of ExpiCHO cells transfected with human GPC3. ExpiCHO cells were transiently transfected with GFP (transfection control) and human GPC3. 100,000 cells were fixed to a 96-well glass bottom plate using 4% PFA at 37C for 15 min. Cells were permeabilized with 0.1% Triton X-100 at RT for 10 min and then blocked with 5% BSA, 5% serum, and 0.01% Triton X-100 at room temperature for 30 min. Cells were incubated with 1 µg/mL Anti-Glypican 3 (GPC3) [IPI-mGPC3.13] (Addgene #222514). Alexa Fluor 647 F(ab')2 fragment goat anti-rabbit IgG, Fc fragment-specific (Jackson ImmunoResearch, 111-606-046) was used as the secondary antibody. Nuclear staining was performed using 0.5 mg/mL DAPI. Cells were imaged for GFP (green), DAPI (blue), and GPC3 (red) using EVOS M7000 microscope at 10x magnification. doi: https://doi.org/10.57733/addgene.3h6ynb

Antibody Details

Antibody design and production

Human variable domains for the heavy and light chain of the FAB fragment used in yeast display were grafted onto the constant CH1, CH2 and CH3 domains of rabbit IgG. The chimera human/rabbit IgG1 construct was recombinantly expressed in ExpiCHO cells, using pTipi2.1 as the expression vector. The antibody was purified by affinity chromatography using protein A (XYZ) and acid elution, followed by immediate buffer exchange using 1 x PBS buffer pH 7.4.

Sequence information

Heavy chain and light chain amino acid sequences are available upon request after purchase. **Contact us** to request.

Antibody Characterization

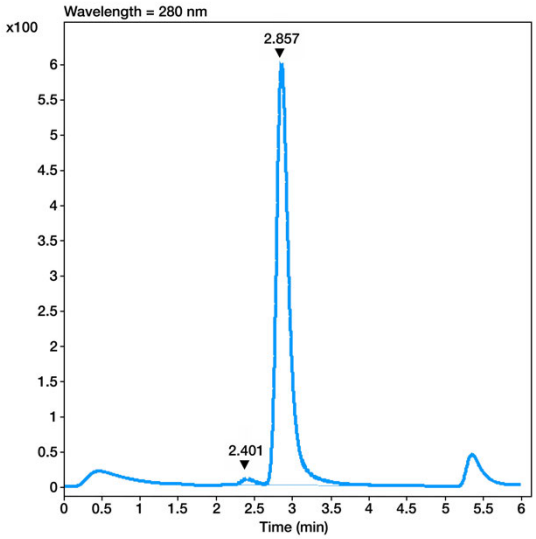
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation of the masses corresponding to the amino acid sequences of both chains.

	HC MW (Da) *Calculated*	HC MW (Da) *Observed*	HC MW (Da) Delta	LC MW (Da) *Calculated*	LC MW (Da) *Observed*	LC MW (Da) Delta
IPI-mGPC3.13	49851.72	49856.70	4.98	22952.44	22951.84	-0.60

Heavy Chain (HC) Mass Calculation: The calculated molecular weight (MW) of the HC is derived by adding the mass of the unmodified HC amino acid sequence to the mass of the predominant N-glycan form (G0F), which is 1444.5 Da. This calculation assumes that the intrachain disulfide bonds remain intact. For HCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), resulting in a deduction of 17.03 Da from the total mass. Additionally, for HCs with a C-terminal lysine (K), the mass of K (128.09 Da) is also subtracted.

Light Chain (LC) Mass Calculation: The calculated molecular weight (MW) of the LC is obtained from the mass of the unmodified LC amino acid sequence, assuming that the intrachain disulfide bonds are not reduced. For LCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), leading to a deduction of 17.03 Da from the total mass. For LCs with a C-terminal lysine (K), the mass of K (128.09 Da) is subtracted as well.

Size Exclusion Chromatography (SEC): SEC is a protein purification technique that separates molecules based on size.



High-performance liquid chromatography-size exclusion chromatography (HPLC-SEC) profile. 5 µg of antibody (1 mg/mL) was analyzed. 1x PBS, pH 7.4 was used as the mobile phase. Protein standards ranging from 15-600 kDa were used for calibration (Sigma, 69385) and Lox1.9 antibody was run as an internal control. Resulting peaks were analyzed using Agilent OpenLab CDS software to determine total peak area and percentage purity.

	Purity	Result
IPI-mGPC3.13	≥95%	Pass

Antigen Details

Immunogen design:

cDNA of Mouse Glypican 3 (GPC3) with C-terminal His-, Avi-, and HA-tags was produced in transiently transfected Expi293F cells and purified from culture supernatant by Ni-NTA affinity purification followed by size-exclusion chromatography.

Immunogen sequences:

Mouse GPC3 (AA: 25-553)
QPPPPPDATCHQVRSFFQRLQPGLKWVPETPVPGSDLQVCLPKGPTCCSRKMEEKYQLTARLNMEQLL
QSASMELKFLIIQNAAVFQEAFEIVVRHAKNYTNAMFKNNYPSLTPQAFEFVGEFFTDVSLYILGSDINVDD
MVNELFDSLFPVIYTQMMNPGLPESVLDINECLRGARRDLKVFGSFPKLIMTQVSKSLQVTRIFLQALNLG
IEVINTTDHLKFSKDCGRMLTRMWYCSYCQGLMMVKPCGGYCNVVMQGCMAGVVEIDKYWREYILSLE
ELVNGMYRIYDMENVLLGLFSTIHDSIQYVQKNGGKLTTTIGKLCAHSQQRQYRSAYYPEDLFIDKKILKV
AHVEHEETLSSRRRELIQKLKSFINFYSALPGYICSHSPVAENDTLCWNGQELVERYSQKAARNGMKNQF
NLHELKMKGPEPVVSQIIDKLKHINQLLRTMSVPKGKVLDKSLDEEGLESGDCGDDEDECIGSSGDGMV
KVKNQLRFLAELAYDLDVDDAPGNKQHGNQKDNEITTSHSGSGHHHHHHHHHHGSGGLNDIFEAQKIE
WHEGSGDYKDDDDK

Sequence information:

HUGO: MGI:104903
Uniprot: Q8CFZ4
Refseq: NM_016697.3

Structural information:

Topology: Glycosylphosphatidylinositol (GPI) Anchored
PDB IDs: 7ZA1, 7ZA2, 7ZA3, 7ZAV
Alphafold: AF-Q8CFZ4-F1

Expression profiles:

Human Protein Atlas ENSG00000147257-GPC3

References

1. Z. Anderson, H. Li, T. Riedel, H Zhu, R. Meijers, and D. Moshinsky. (2024). Flow Cytometry for Anti-Glypican 3 (GPC3) [IPI-mGPC3.13]. Addgene. https://doi.org/10.57733/addgene.ohyqj9
2. Z. Anderson, H. Li, T. Riedel, H Zhu, R. Meijers, and D. Moshinsky. (2024). Flow Cytometry for Anti-Glypican 3 (GPC3) [IPI-mGPC3.13]. Addgene. https://doi.org/10.57733/addgene.jj5ts7
3. H. Li, Z. Anderson, T. Riedel, and D. Moshinsky. (2024). IF for Anti-Glypican 3 (GPC3) [IPI-mGPC3.13]. Addgene. https://doi.org/10.57733/addgene.3h6ynb
4. A. Salic. (2024). Immunofluorescence for Anti-glypican 3 (GPC3) [IPI-mGPC3.13]. Addgene. https://doi.org/10.57733/addgene.9krw4c
5. J. Trimmer. (2024). IHC for Anti-Glypican 3 (GPC3) [IPI-mGPC3.13]. Addgene. https://doi.org/10.57733/addgene.qjafi3

How to cite this antibody:
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